Infusing structural assumptions into dimension reduction for single-cell RNA sequencing data to identify small gene sets

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Abstract

Dimension reduction greatly facilitates the exploration of cellular heterogeneity in single-cell RNA sequencing data. While most of such approaches are data-driven, it can be useful to incorporate biologically plausible assumptions about the underlying structure or the experimental design. We propose the boosting autoencoder (BAE) approach, which combines the advantages of unsupervised deep learning for dimension reduction and boosting for formalizing assumptions. Specifically, our approach selects small sets of genes that explain latent dimensions. As illustrative applications, we explore the diversity of neural cell identities and temporal patterns of embryonic development.

Keywords: *Dimension reduction, Deep learning, Variable selection, Interpretability, Explainable artificial intelligence, Differentiable programming, Single-cell RNA sequencing data, Time series*

1 Background

Dimension reduction approaches are an essential component of exploratory single-cell RNA sequencing (scRNA-seq) data analysis to identify underlying patterns of cellular heterogeneity, e.g., by providing visualizations in two dimensions [1, 2]. Such low-dimensional representations are then often used for further downstream analyses to answer more targeted questions including cell clustering and trajectory inference [3, 4, 5]. However, it might be beneficial to already encode additional assumptions directly into the dimension reduction, to find a representation that is specifically tailored to structural knowledge or intuition about a dataset, e.g., corresponding to an intuition

about expected patterns or knowledge about experimental design. For example, researchers might expect groups of cells to be captured in different dimensions, which are each characterized by a distinct, small set of genes. When collecting data at different time points during a developmental process, researchers may seek to identify sets of genes that explain the heterogeneity at each time point, but in particular also changes between time points. This requires a flexible approach that can be adapted to incorporate different types of assumptions, such as about experimental design or expected structure. To achieve this, we propose to combine dimension reduction with variable selection, where assumptions are formulated as constraints. For example, a modeler could decide that only genes that are complementary to the information encoded in the other dimensions should be selected for a particular reduced dimension. This would ensure that different dimensions capture distinct factors of variation, corresponding to different cell groups, to provide a targeted understanding of cellular heterogeneity.

Currently, non-parametric approaches such as t-SNE [6, 7] and UMAP [8, 9] are the most widely used to perform dimension reduction of omics data for visualization, due to the resulting visually appealing, fine-grained two-dimensional representations. As they are optimized in a fully unsupervised, algorithmic manner, it is not straightforward to encode additional constraints and link specific patterns in the low-dimensional space to individual explanatory genes. While this could in principle be addressed in corresponding parametric alternatives (e.g., [10, 11, 12, 13, 14, 15]), where a mapping to the latent space is learned as an explicit function specified by a neural network, it may then be more attractive to directly use neural network-based approaches for dimension reduction.

Specifically, autoencoders and variational autoencoders [16] have been used to learn neural network-based low-dimensional latent representations of single-cell transcriptomics (e.g., [10, 17, 18, 19]), multi-omics [20, 21], or spatial transcriptomics data [22]. Autoencoder-based models have also been adapted to encode additional structure in a latent representation, e.g., using external data in single-cell applications [23, 24, 25], or to specifically provide sparse [26] or disentangled latent representations [27, 28, 29]. However, such approaches are typically designed to satisfy a single specific constraint, and cannot be flexibly adapted to encode different types of structure in a straightforward manner [30]. Since their optimization often involves several additional penalty terms that must be carefully calibrated in the overall loss function, such models tend to be quite complex even for a single constraint. Another drawback of deep neural network models is their natural lack of interpretability due to architectures that typically include multiple nonlinear layers. While multiple layers for dimension reduction allow for more flexibility in the learning task, which can help to construct a well-structured embedding of the data in a latent space, it is particularly difficult to determine the most explanatory genes for the learned patterns in different latent dimensions. Therefore, neural network-based approaches often rely on post-hoc feature attribution to link groups of genes to specific latent dimensions [31, 32, 33], where an additional analysis step is applied to an already trained model (e.g. [34, 35]). Still, there are some approaches that incorporate interpretability already as part of the model design. For example, the siVAE approach simultaneously infers a cell and gene embedding space via two encoder-decoder frameworks and and uses an additional regularization term in the loss function, where embeddings of genes indicate their contribution to distinct dimensions of the cell embedding space [36]. However, the dimensions in the cell embedding may still be entangled, and the contribution of features to the dimensions of the cell embedding is not constrained to be sparse.

Similarly, simpler dimension reduction approaches, such as principal component analysis (PCA), allow direct inspection of the contribution of individual variables to any latent dimension [37, 38],

but again provide a dense representation instead of distinct, small sets of genes associated with each dimension. Approaches based on L_1 penalties have been proposed to enforce sparsity, i.e., to obtain selection of gene sets [39, 40], but these do not provide the dimension reduction flexibility of neural networks.

While neural networks can in principle be combined with L_1 penalties (e.g. [41, 42]), we propose to use a more flexible approach for variable selection, namely componentwise boosting [43, 44], which has been successfully applied in various omics applications (e.g., [45, 46, 47, 48]). This approach provides variable selection and regularization similar to L_1 penalties [49]. However, it relies on separate variable selection and parameter update components. The selection component can be flexibly adapted (e.g., [50]). When combined with neural networks for dimension reduction, this could allow to incorporate structural assumptions, e.g., such as each latent pattern being associated with a small set of explanatory genes, or such as the presence of gradually evolving differentiation trajectories, or distinct groups of cells.

Yet, end-to-end models with componentwise boosting and neural networks are not available so far. The challenge in this is that parameters in autoencoder neural network architectures are trained by minimizing a reconstruction loss via stochastic gradient descent in parameter space, while boosting approaches rely on gradients in function space [51]. To overcome this challenge, we propose the boosting autoencoder (BAE) approach. Specifically, we use the gradient of the reconstruction loss backpropagated through a decoder component of an autoencoder to obtain an outcome criterion for then building the encoder part via componentwise boosting. Joint optimization in an end-to-end model is facilitated by differentiable programming, a paradigm in which gradients of a joint loss function for simultaneous optimization of potentially diverse model components are obtained via automatic differentiation [52, 53].

Due to the sparsity constraint, the proposed BAE approach should allow to find small gene sets that characterize very small cell groups with a distinct transcriptomic signature, which might be lost in a global clustering [54]. In contrast to the end-to-end approach we are aiming for, standard analyses to characterize different cell groups by marker genes typically correspond to two-step approaches, where clustering and subsequent analysis by post-hoc differential gene expression tests are performed separately [55, 56]. Even if a very small group is captured by such a clustering, the statistical power to infer marker genes is often limited due to the small sample size [57, 58]. Dedicated approaches have been developed to address this problem (e.g., [59, 60, 58]). However, these approaches are specifically tailored to this task, whereas the proposed BAE approach should provide a more general method for encoding different constraints.

We specifically evaluate the performance of the proposed BAE approach with respect to capturing distinct cell types in different latent dimensions while simultaneously identifying sparse sets of corresponding marker genes in an application on neurons from the primary visual cortex of adult mice [61], including the analysis of very small groups of cells. We also investigate an adaptation to time-resolved scRNA-seq data, where the aim is to capture developmental patterns in different latent dimensions along with characterizing gene programs, i.e., to infer gene expression dynamics despite no one-to-one correspondence between cells at different time points.

An implementation of our approach, including code to reproduce all results and a tutorial notebook, is available at `https://github.com/NiklasBrunn/BoostingAutoencoder`.

2 Results

2.1 Combining componentwise boosting and neural networks for incorporating structural assumptions into dimension reduction

To learn a representation that reflects intuition about underlying structure or experimental design, we have developed the boosting autoencoder (BAE) approach. Dimension reduction by an autoencoder is adapted by replacing the encoder by a componentwise boosting approach, which serves to identify a sparse set of genes characterizing each latent dimension and to formalize constraints via the boosting step selection criterion. Figure 1 illustrates the overall model architecture, including constraints for disentangled latent dimensions to reflect different cell groups (middle row) and differentiation trajectories via coupling time points (bottom row) as exemplary applications. The normalized gene expression count matrix is mapped to a latent space via a boosting encoder, which in the simplest case corresponds to a linear transformation by a sparse weight matrix. The encoder can be conceptualized as a linear neural network (i.e., without activation function and bias term) or, alternatively, as a set of generalized linear models (one for each latent dimension) with an identity link function. Its parameters are fitted via componentwise boosting [43] (see Methods Section 5.3). The decoder, corresponding to a neural network, maps the latent representation to a reconstruction of the input data (see Methods Section 5.2). Its parameters are optimized via stochastic gradient descent [62] on the reconstruction loss.

In boosting, optimization aims to minimize an outcome-based criterion. To obtain such a criterion, we use the negative gradients of the autoencoder reconstruction loss with respect to the latent representations as responses for the variable selection, i.e., we couple variable selection and optimization of the autoencoder (see Methods Section 5.4). The negative gradients correspond to the direction in which the latent representation needs to be changed in order to minimize the reconstruction loss. Thus, genes are selected that are most strongly associated with these directions, ensuring that variable selection aligns with finding an optimal dimension reduction. Importantly, the variable selection enforces a sparse mapping to the latent space where only a small number of explanatory variables, i.e., genes, are linked to each latent dimension, facilitating a biological interpretation. The BAE is thus implicitly regularized by the boosting.

The selection criterion can be flexibly customized by incorporating constraints into parameter estimation, corresponding to structural assumptions. Specifically, we developed a disentanglement constraint, where the selection in each dimension almost only considers genes that explain complementary information to that already encoded in other dimensions (Figure 1, middle row). The approach thus captures distinct sets of genes that characterize the expression patterns of cells in different dimensions (see Section 2.2), which may, e.g., represent different subgroups of cells. As another example of the flexibility afforded by the boosting component, we developed a constraint for encoding experimental design knowledge on gradually evolving gene expression dynamics in time series data (Figure 1, bottom row and Methods Section 5.7). Specifically, we train a BAE with the disentanglement constraint at each time point and pass the weight matrix between time points, such that optimization for a given time point respects the changes from the previous time point, and dimensions that reflect the same development pattern across time points are coupled. This is to identify developmental trajectories and corresponding gene programs (see Sections 2.4 and 2.5).

During training, the boosting component updates selected weights with either a positive or negative value, allowing the model to use the entire latent space to learn a well-structured data representation. After training, the gene sets driving each latent dimension can be extracted by

inspecting the top nonzero coefficients of the encoder weight matrix. We determine the top genes for each dimension by normalizing each weight corresponding to a gene by the maximum of the absolute values of the other weights for that dimension. In addition, the representations of cells in particular dimensions can be examined by comparing the signs of the representation values with the signs of the normalized coefficients corresponding to the top-selected genes for that dimension. Specifically, if a cell group is highlighted in a latent dimension with positive activation values, the top genes with a corresponding positive weight are characteristic for that group. To simplify the analysis of the learned patterns in distinct latent dimensions, we suggest using a two-dimensional UMAP embedding of the BAE representation of cells, colored individually by the representation values of the cells per dimension, for a visually easy-to-interpret comparison. The analysis of gene sets and corresponding latent patterns can be used to elucidate underlying biological mechanisms, e.g., for de novo cell type identification and annotation or to identify developmental patterns. In addition, the learned latent representation serves as a basis for standard downstream analyses (discussed further in Section 2.2).

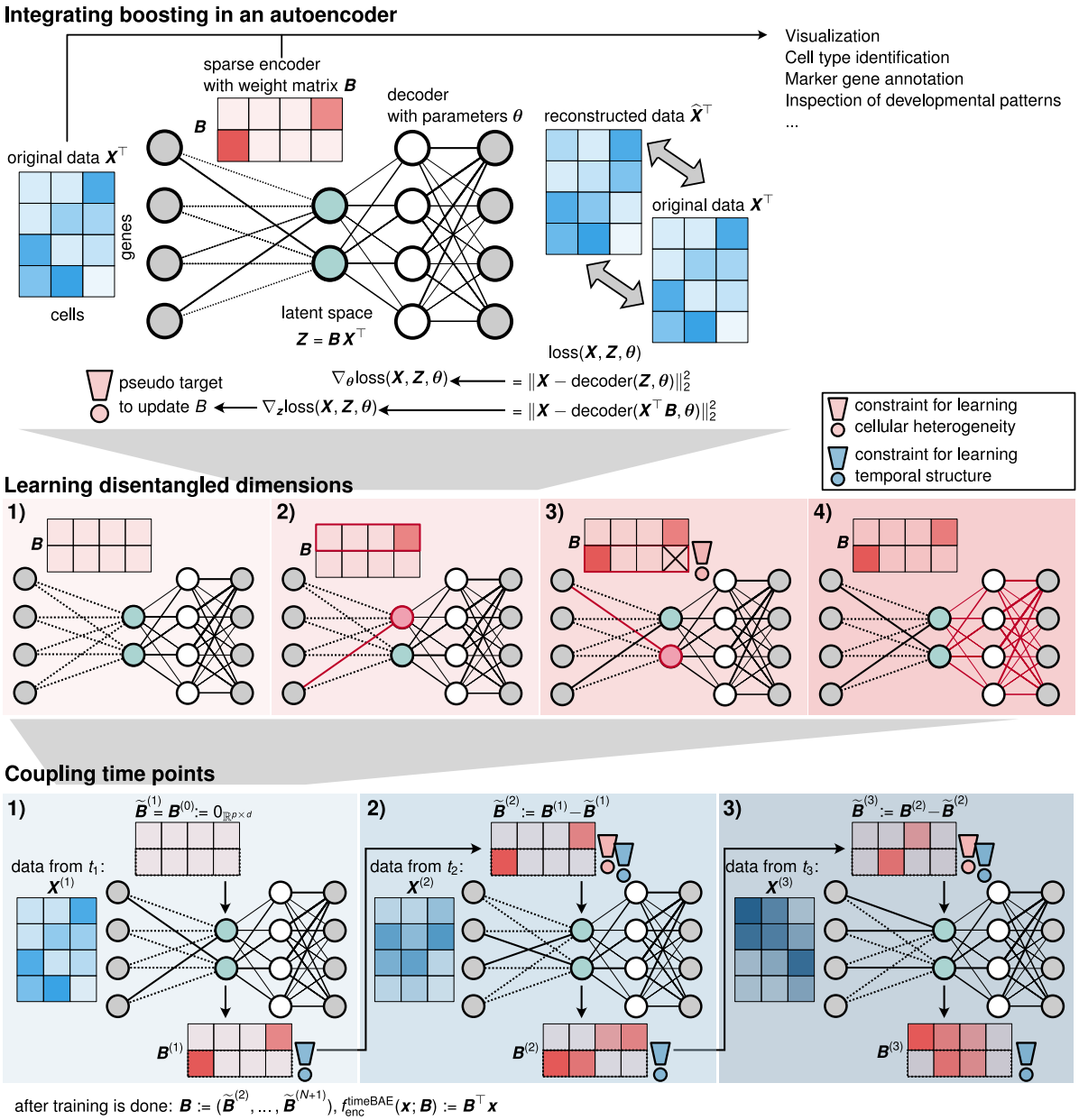

Figure 1: **Top row: Overview of the boosting autoencoder (BAE) architecture.** A gene-by-cell matrix is mapped to a low-dimensional latent space via multiplication by a sparse encoder weight matrix. The decoder infers a reconstruction of the original data and is optimized by gradient descent on the reconstruction loss. The encoder weights are fitted via componentwise boosting, using the negative gradients of the reconstruction loss with respect to the latent representation as responses. Estimation of corresponding model parameters can be constrained to encode additional assumptions (illustrated by the exclamation mark). Training of the encoder and decoder can be performed simultaneously by differentiating through the boosting step. After training, a latent representation is obtained by multiplying the data X with the sparse encoder weight matrix B for downstream analysis.

Middle row: Training process with a constraint for disentangled latent dimensions. The encoder weights are initialized as zero (1), and for the first latent dimension, one coefficient of B is updated to a nonzero value (2). In all subsequent updates, only variables complementary to the ones already selected may be selected (3, constraint indicated by the exclamation mark). After updating one coefficient for each dimension, the decoder parameters θ are updated via gradient descent (4). Steps (2)-(4) are repeated in subsequent training epochs.

Bottom row: Adding a constraint for coupling time points. For extracting differentiation trajectories from time series scRNA-seq data, a BAE is trained at each time point (1-3). The encoder weight matrix, trained with the disentanglement constraint (red exclamation mark), is passed to the subsequent time point in a pre-training strategy (blue exclamation mark), to couple dimensions corresponding to the same developmental pattern across time (indicated by the dashed box around the second dimension).

2.2 The BAE identifies cell types and corresponding marker genes in cortical neurons

We apply the BAE to a scRNA-seq dataset from [61] of 1,679 neurons of mice from the primary visual cortex (see Methods Section 5.11 for details on the dataset and preprocessing). The dataset comprises several neuronal subpopulations that have been characterized by the original authors and annotated with corresponding marker genes ([61], Figure 3 and Supplementary Material, Figure S15). Our goal is to recover these subpopulations in different dimensions and simultaneously infer a small set of genes for each dimension, which should include marker genes of the corresponding cell type. Accordingly, we tailor the selection criterion in the boosting component of the BAE to select only genes for each dimension are that carry complementary information to the genes already selected in other dimensions (see Methods Section 5.5) to ensure that different dimensions represent subpopulations with distinct molecular signatures.

After preprocessing, the data matrix contained log1p-transformed normalized counts of 1,500 highly variable genes in 1,525 cells, including neural cell type marker genes. We randomly sampled 1,325 cells as the training dataset, used the remaining 200 cells as test set, and standardized both datasets. We trained a BAE with a 2-layer decoder and 10 latent dimensions (see Methods Section 5.6 for details). Note that the cell type and marker gene annotations from [61] were not provided to the model, but were subsequently used as ground truth to evaluate the identified patterns.

Figure 2a shows a heatmap of the latent representation of all cells after training, sorted and colored by annotated cell types together with a 2D UMAP embedding of the BAE representation of the cells. Each dimension captures a specific group of cells that closely match some of the annotated cell types. In the UMAP plot, cells of the same type cluster together, further confirming that the BAE has captured cell type-characterizing patterns in its latent dimensions.

To highlight learned patterns in specific latent dimensions, we color each cell in the UMAP embedding according to the value of its representation in a BAE latent dimension (Figure 2b). A high absolute value for a cell in a particular dimension means that the genes associated with that dimension with a sign matching the actual representation value are characteristic of that particular cell. We can thus visualize the patterns captured in each latent dimension (see Figure 2b for dimensions 1-5 and Supplementary Figure S1 for dimensions 6-10). Each dimension captures a group of cells that mostly corresponds to a specific annotated cell type. Inspection of the corresponding selected genes shows that the top genes for each dimension are among the listed neural marker genes of the respective cell types (Figure 2c and Figure 3 in [61]). In addition, cell type predictions on the held-out test data are accurate, indicating that new data can be projected onto the learned representation via the encoder and annotated according to its dominant patterns (results not shown).

We also applied the underlying boosting algorithm (compL_2Boost, see Methods Section 5.3) in a supervised mode using annotated cell type labels as responses. Comparison of the latent BAE patterns with the predictions from the supervised compL_2Boost showed that the BAE identifies very similar patterns despite being unsupervised (see Supplementary Section S2 and Supplementary Figure S2).

The BAE approach is also capable of capturing and characterizing small subgroups of cells. For example, in our application, it identifies two distinct groups of *Sst*-expressing neurons in dimensions 4 and 5, and simultaneously provides candidate marker genes via the sparse gene sets associated with the corresponding dimensions (Figure 2b, c; more details on the *Sst*-groups in Section 2.3). In addition, we noticed that across several training runs with different decoder parameter initializations and model hyperparameters, one of the latent dimensions consistently captured the

same small group of cells of various annotated types, here represented in latent dimension 8 (see Supplementary Figure S1). The corresponding scatter plot of the top genes suggests *Gpr17* and *Cd9* as characteristic of that group. While *Cd9* is listed as a transcriptomic marker of oligodendrocyte precursor cells in the original study of cells from the primary visual cortex of mice (see Figure 3 in [61]), the receptor Gpr17 has been identified as a a regulator of oligodendrocyte development (see, e.g., [63, 64]). However, the corresponding cells were classified as different neurons in [61]. The BAE can thus help to infer cell identities of even very small groups of cells based on a sparse set of characteristic genes.

While cell identities are often determined in a two-step process, where clustering of cells is followed by marker gene selection, e.g., based on differential expression, the BAE can simultaneously identify groups and a corresponding sparse gene expression signature in a one-step approach. Because it does not rely on predefined cell clusters or types and can capture very small groups of cells, it can identify misclassified cells, help to determine the identity of unclassified cells or investigate cell types at a finer granularity.

As shown so far, the BAE allows to encode expected structural assumptions about the presence of different subpopulations of neurons with different sets of transcriptomic markers. Additionally, the approach facilitates biological interpretability by allowing to identify distinct patterns of gene expression heterogeneity, to detect cell group-specific gene sets even for small cell groups, and to confirm or potentially correct previous annotations.

To demonstrate that the BAE can be used as an alternative dimension reduction also for subsequent downstream analyses in a standard scRNA-seq analysis workflow without loss of performance, we exemplarily compared visualization and clustering results based on a BAE and as an alternative to a PCA representation. Specifically, UMAP embeddings of a BAE representation show a very similar structure to the UMAP based on the PCA representation, grouping together cells of the same type (Supplementary Figure S3b). In addition, we performed Leiden clustering based on the BAE and PCA representations and computed silhouette scores and adjusted Rand indices for 20 clustering results for each approach (see Methods Section 5.8 for details). Specifically, we re-trained the BAE with a different random initialization of the decoder parameters for each clustering. The median silhouette score for the BAE clusterings is 0.41 and the median silhouette score for the PCA clusterings is 0.43, i.e., there is no substantial difference in performance when taking into account the variability of the BAE due to random initializations (interquartile range of 0.03). The median adjusted Rand index between the two clusterings is 0.82, confirming that the BAE representation provides competitive clustering performance.

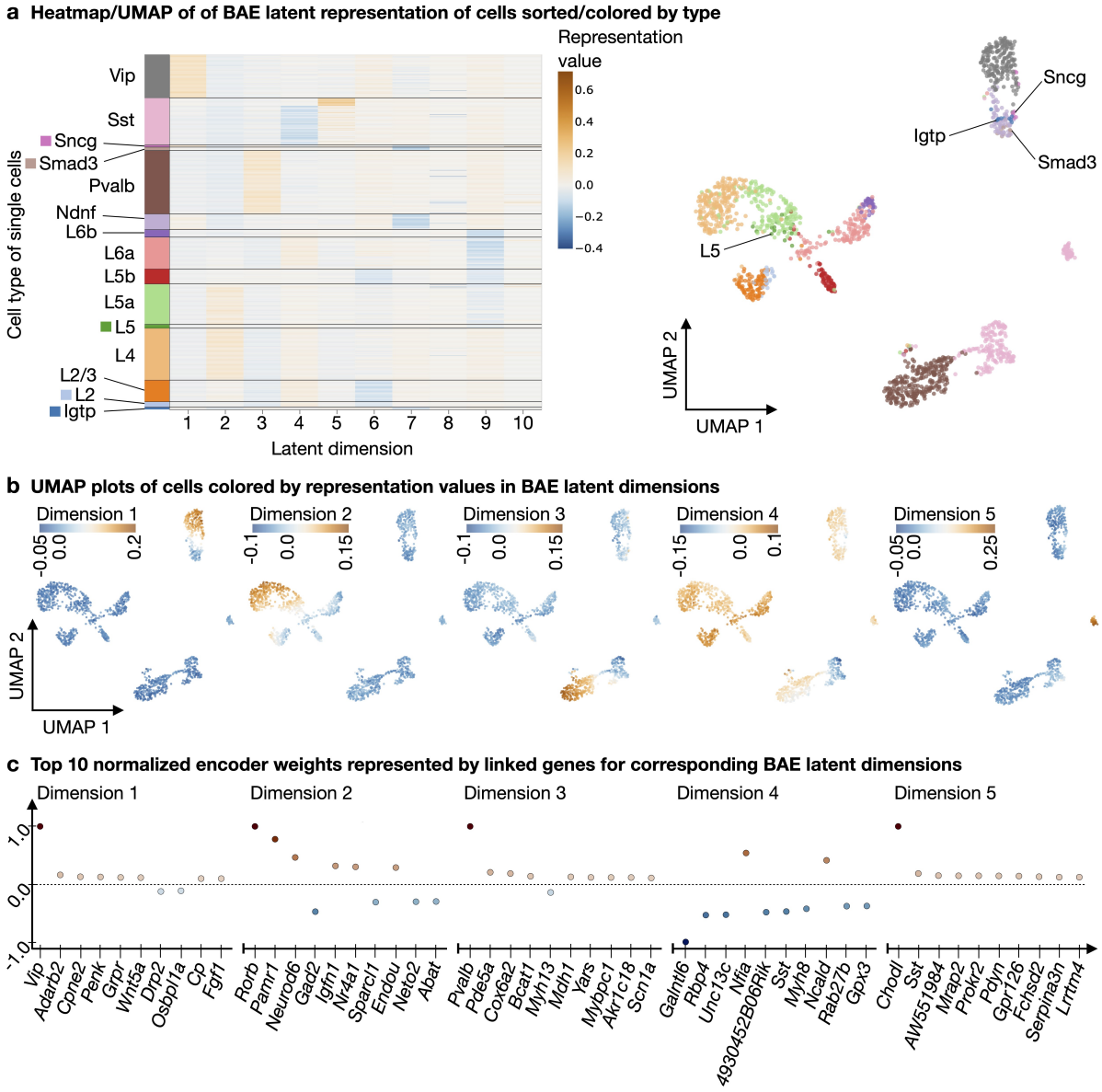

Figure 2: **BAE analysis on cortical mouse scRNA-seq data. a** Left: Heatmap of a sparse BAE latent representation of cortical neurons sorted by cell type. Right: Scatter plot of a 2D UMAP embedding of the sparse representation colored by types. **b** Scatter plots of the same UMAP embedding, colored by the representation values of cells in individual BAE latent dimensions. The first five dimensions are shown. **c** Scatter plots show the top ten normalized nonzero BAE encoder weights (represented by linked genes) in decreasing order of absolute value (from left to right) per latent dimension. The first five dimensions are shown. Normalization was performed by dividing each weight by the maximum absolute weight value for the corresponding latent dimension.

2.3 Subgroup analysis of cortical neurons with a small number of cells

Motivated by the fact that the BAE was able to detect a subset of *Sst*-expressing neurons characterized by the neural marker *Chodl*, we singled out the *Sst*-expressing neurons for a more fine-grained detection of *Sst*-expressing subgroups. This is to demonstrate that the BAE can be useful even with a very small number of cells. Here, subgroup detection is challenging because there are only very few *Sst*-neurons, namely $n = 202$. Also, cells of the same type tend to be more homogeneous in their expression profile and, thus, thus may be more difficult to disentangle. To indetify subgroups, we select the 1,500 most highly variable genes on the subpopulation and train a BAE using the disentanglement constraint with four latent dimensions for 20 epochs.

After training, we again inspect the learned patterns based on UMAP plots of the latent embedding, colored by the representation values in different latent dimensions, together with scatter plots of the corresponding selected top genes for the dimensions (see Figure 3a,b). For comparison, we computed a UMAP embedding based on a PCA representation, which does not identify distinct clusters as well (see Figure 3c). Examining the patterns learned in different latent dimensions of the BAE via the colored UMAP plots shows that the approach captures distinct small subgroups of cells. Inspection of the sample Pearson correlation coefficients between the latent dimensions confirms that the learned dimensions of the BAE are uncorrelated, thus different cell group characterizing genes were associated with different dimensions (see Figure 3d). By further analyzing the top-selected genes shown in the scatter plots we found that some of the top genes corresponded to the genes listed in [61] Figure 3a for subgroups of the *Sst*-neurons. For example, the subgroup of *Sst*-neurons characterized by the gene *Chodl* is captured in dimension 4 and clearly separated from the remaining cells. Also, for dimension 1, *Myh8* was the top-selected gene characterizing a subgroup at the top center of the UMAP plot. In dimension 2, the top-selected gene is *Th*, which characterizes a small group of cells in the center of the UMAP plot.

2.4 Incorporating more complex structural knowledge: timeBAE for temporal patterns

To demonstrate how the BAE approach can be flexibly tailored to different data structures via the variable selection criterion, we investigate an application on simulated time series scRNA-seq data. The goal is to 1) identify distinct developmental patterns at each time point, driven by corresponding sets of genes, and 2) link latent dimensions that capture the same developmental pattern at different time points. This will then allow the identification of distinct trajectories across time points, despite the lack of one-to-one correspondence between cells at different time points. We assume that multiple differentiation trajectories may be present in the data. Specifically, we expect the presence of different groups of cells at each time point, where each group at one time point may be linked to another group at the following time point. Linked groups are characterized by some similar, but also some novel gene expression patterns, i.e., there is some overlap in the characterizing gene sets at subsequent time points, corresponding to the gradual up- and downregulation of genes during development. We illustrate how these assumptions can be incorporated into the BAE approach, and show how this adaptation can be evaluated by a corresponding simulation.

We simulate data to reflect developmental processes of three groups of cells across three time points. Each group is characterized by a distinct, small set of genes at each time point, with some overlap of genes from linked groups at two subsequent time points, as shown in Figure 4a. Specifically, we generate binary counts indicating high vs. low expression of a gene, similar to the simulation design in [35]. Each group is characterized by 8 highly expressed genes, 3 of which are

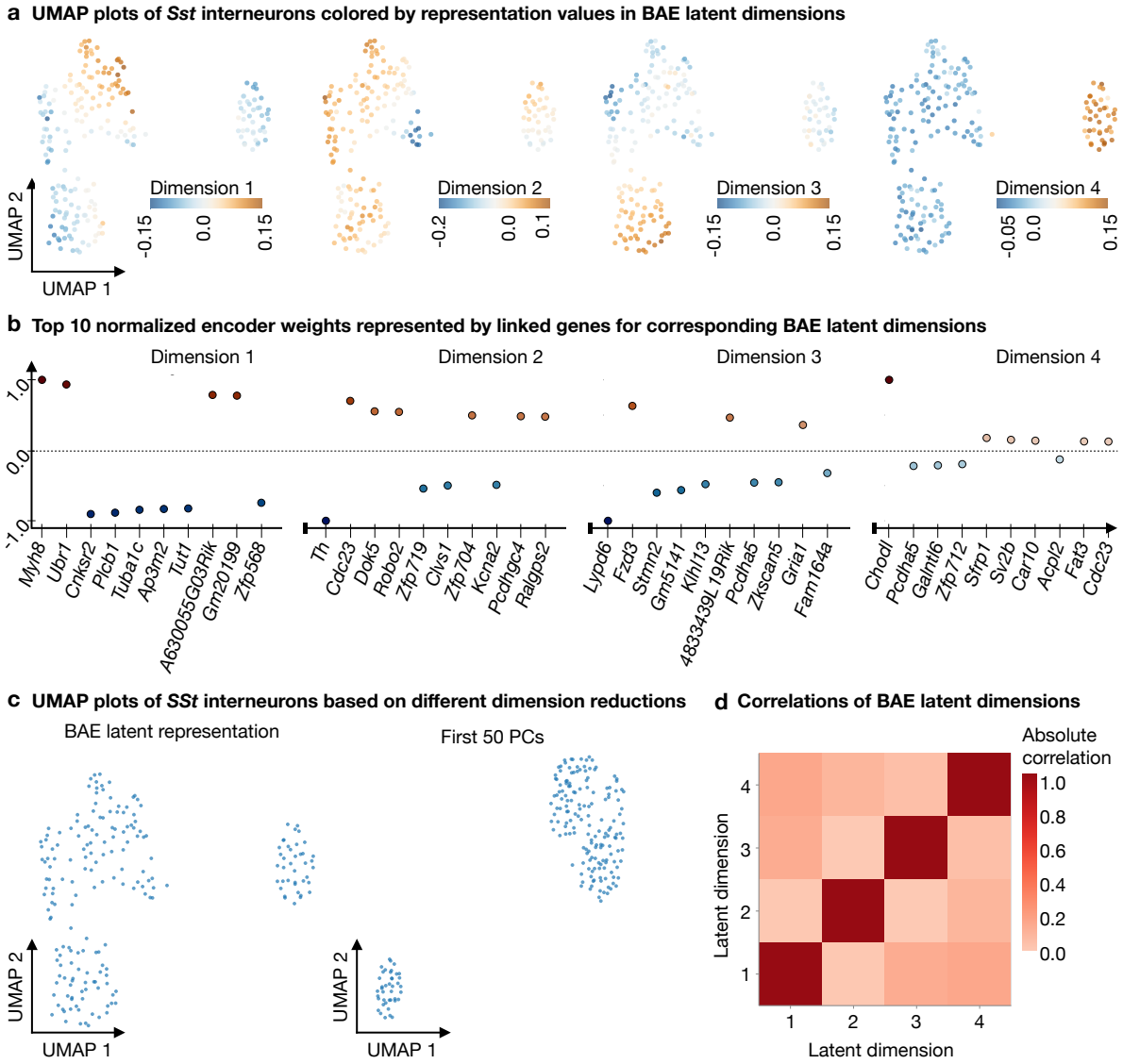

Figure 3: **BAE subgroup analysis of *Sst*-expressing neurons from mouse cortex.** **a** Scatter plots of a 2D UMAP embedding based on a 4D BAE latent representation of *Sst*-neurons, colored by the representation values of the cells in each dimension. **b** Scatter plots showing the top ten normalized nonzero BAE encoder weights (represented by linked genes) in decreasing order of absolute value (from left to right) per latent dimension. Normalization was performed by dividing each weight by the maximum absolute weight value for the corresponding latent dimension. **c** Left: Scatter plot of the 2D UMAP embedding calculated using the 4D BAE latent representation. Right: Scatter plot of a 2D UMAP embedding calculated using the first 50 principal components resulting from PCA of the *Sst*-neurons. **d** Heatmap shows the absolute values of the Pearson correlation coefficients between the representations of cells in the BAE latent dimensions.

also highly expressed in the subsequent group of cells along the same differentiation trajectory at the next time point (e.g., genes 1-8 are characteristic for group 1 at time point 1, genes 6-13 for group 1 at time point 2, genes 11-18 for group 1 at time point 3, etc.). In addition to the in total

54 trajectory-characterizing genes, we simulate 26 non-informative noise genes. The simulated expression value for each cell is generated by sampling from a Bernoulli distribution with a higher probability of success for a highly expressed gene, and a lower probability of success for a lowly expressed gene (see Methods Section 5.10 for details). For our analysis, we generated count matrices consisting of 310 cells at the first time point, 298 cells at the second time point, and 306 cells at the third time point, equally dividing the cells among the three groups.

To not only infer the characterizing genes for each group and time point separately but also to identify the groups of cells that overlap in their gene sets, we adapt the BAE to couple dimensions across time points. This allows the identification of cells that belong to the same differentiation trajectory, knowing that an overlap in gene sets does not exclusively correspond to cell differentiation. Specifically, we train one BAE for each time point, using the samle number of dimensions for each model (see Methods Section 5.7 for details). For example, assuming three distinct groups at three time points, we train three BAEs with three latent dimensions each.

The overall approach, which we call timeBAE, is optimized by successively training each BAE with the data from one time point. While the decoders are randomly initialized, the encoder weights for a given time point are initialized with the learned encoder weights from the previous time point. That is, when we optimize the encoder at one time point, we simultaneously pre-train the encoder of the following time point, as shown in Figure 1. Thus, we guide the BAE of each time point to preferentially select variables related those already included in the same latent dimension at a previous time point, in order to capture gradually evolving gene patterns that characterize distinct developmental trajectories. For the final latent representation, we concatenate the representations of the individual BAEs. The overall approach reflects cell group-characterizing patterns at each time point, while tracking the developmental processes of these groups via the correspondence of latent dimensions across time. The approach is compatible with both the alternating and the joint training mode explained in Methods Section 5.4.

The results for training a timeBAE with three latent dimensions for each BAE at each time point on the simulated data are shown in Figure 4. Panel a shows heatmaps of the final BAE encoder weight matrices after training on the data at every single time point. We designed the timeBAE such that the same latent dimension in each individual BAE represents the same cell lineage over time. Sparse sets of selected genes for each dimension correspond to the simulated developmental patterns of highly expressed genes over time, shown by the vertical dashed lines in Figure 4a. A direct comparison of the whole binary count data with its latent representation by the timeBAE encoder further shows that the sparse patterns of the representation match the patterns in the simulated binary count data (Figure 4b). In particular, cells in a latent dimension that encodes patterns of a specific time point in a lineage are slightly activated when the cells correspond to measurements from the previous or subsequent time point. In addition, the correlation patterns of the latent representation in Figure 4c show that almost all latent dimensions representing the same developmental process share a higher absolute Pearson correlation coefficient compared to other dimensions.

2.5 Identification of developmental patterns in real time series scRNA-seq data

In a further experiment, we apply the timeBAE approach to a real time series scRNA-seq dataset of human embryonic stem cells grown as embryoid bodies over a period of 27 days, where differentiation into different cell lineages can be observed [65]. Cells were sequenced during 5 consecutive time intervals, labeled by time points 1-5.

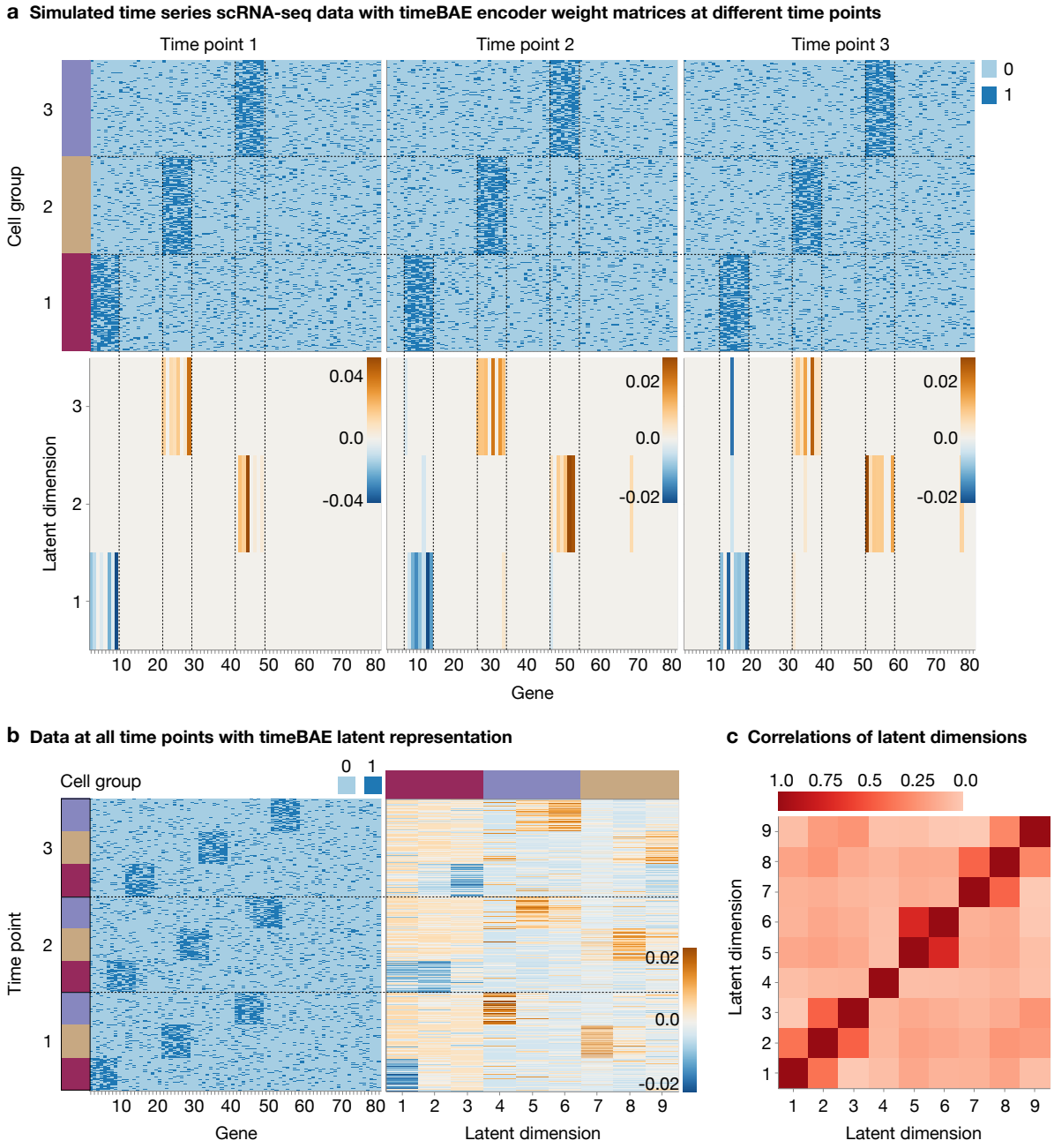

Figure 4: **Investigation of the timeBAE approach on simulated dichotomized scRNA-seq data.** **a** Top row: Heatmaps show the simulated binary count matrices mimicking three lineages of cells across three different time points. Bottom row: Heatmaps illustrate the learned timeBAE encoder weight matrices per time point. **b** Heatmaps of the the stacked binary count data of all time points and the timeBAE latent representation. **c** Heatmap of the absolute values of sample Pearson correlation coefficients between the timeBAE latent dimensions.

We aim to capture sparse gene sets associated with different latent dimensions that may reflect potential markers of different cell stages in a cell lineage. To validate our approach, we consider

a subset of the data where the learned patterns in the linked dimensions can be easily visualized and compared to an embedding that reflects both cluster and temporal structure. Finding such an embedding, where both temporal structure and cluster identity is simultaneously visualized in 2D, is challenging [65]. Therefore, we trim the dataset to retain only cells that can be grouped into large clusters, with a temporal gradient of cells from different time points within each cluster (see Methods Section 5.11). After preprocessing, we ended up with 11,628 cells from 3 different time points with 3 different annotated cluster labels and 263 pre-selected genes for subsequent analysis. Figure 5a shows a 2D UMAP embedding of the trimmed and clustered dataset, which allows to visualize both cluster and time structure.

Next, we train a timeBAE with a 2-layer decoder for each BAE at the different time points using the disentanglement constraint. Each BAE is defined to have four latent dimensions. Thus, the timeBAE has a total of 12 dimensions. After training, we rearrange the dimensions such that linked dimensions corresponding to different time points are adjacent to each other to facilitate the interpretation of the visualized results.

In Figure 5b, we show exemplary UMAP plots colored by timeBAE latent dimensions 1-3 after reordering, where dimension 1 corresponds to the learned patterns from the first time point, dimension 2 corresponds to the second, and dimension 3 corresponds to the third. Comparing the learned patterns with the original cluster and time of measurement information shows that the timeBAE approach captured genes that characterize temporal subgroups of cluster 3 in the correct order. The corresponding set of genes, sorted by importance, is shown in Figure 5c and could provide marker gene information for different cell stages in a cell lineage. However, the learned patterns did not match the cluster and time point patterns from Figure 5a for every set of linked dimensions (Supplementary Figure S4 and S5). In general, the subsequent analysis of temporal patterns in cells potentially revealed by the timeBAE approach should be carefully performed based on the top selected genes for the linked dimensions. This typically requires prior biological knowledge of known markers of cell lineages.

In such a setting, where both temporal structure and cluster identity can be identified in a 2D visualization, we can compare the timeBAE results to a linear regression analysis where we use the time point and cluster annotations to determine the most significant genes and compare them to the top genes selected by the timeBAE. We determined significant genes in the regression analysis by coefficients with the smallest adjusted p-values after correction for multiple testing. Each set of significant genes for each cluster at each time point had multiple overlaps with a set of top selected genes corresponding to a latent dimension of the timeBAE, confirming the importance of the top selected genes for time point-related patterns per cluster (results not shown).

In addition, the results suggest that the timeBAE approach can also be applied in a scenario where the developmental processes of cells cannot be easily visualized in a 2D space. The approach would then still be able to link characterizing gene sets to related latent dimensions that could be used for further post-hoc analysis of lineage relationships.

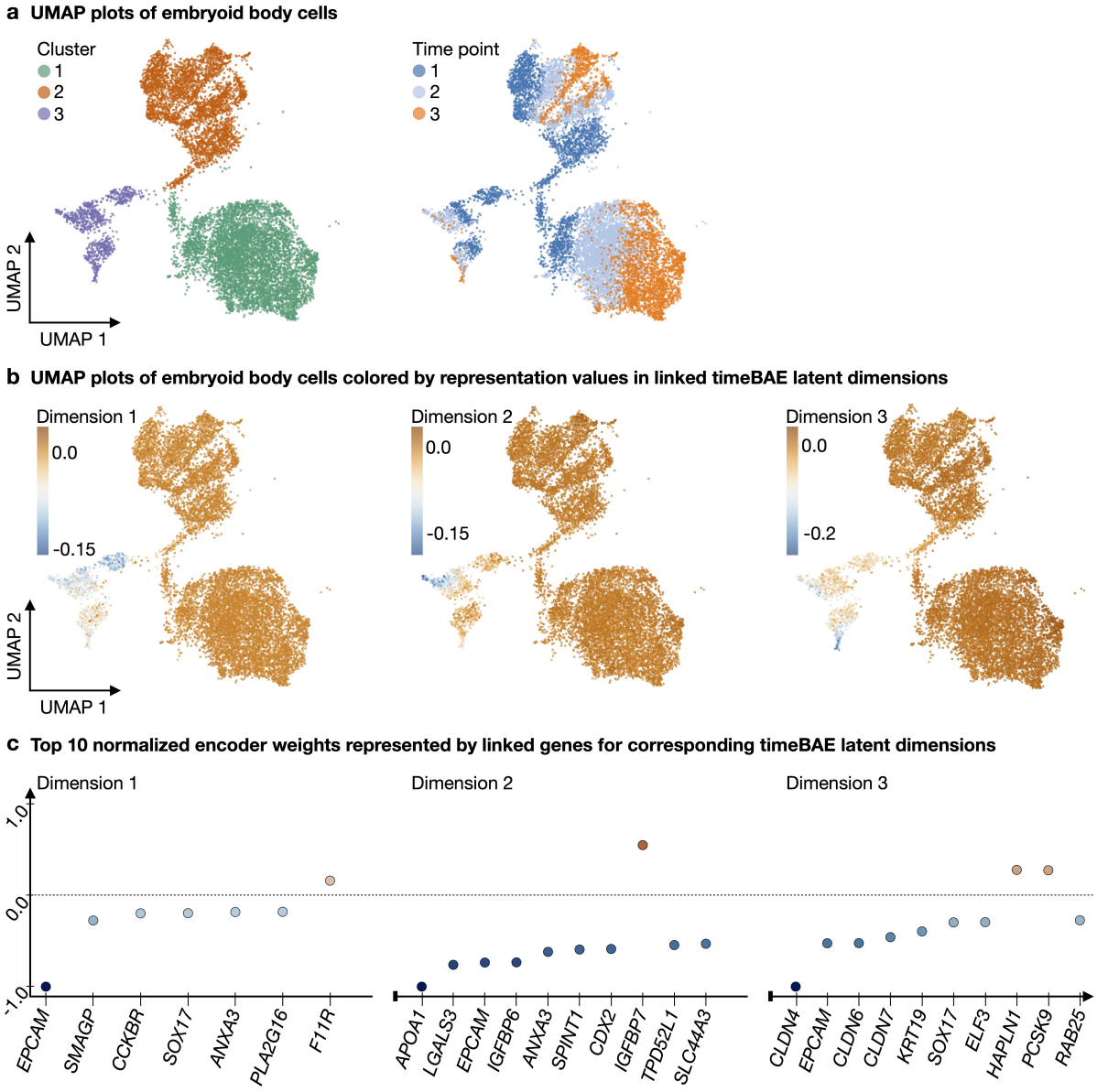

Figure 5: **Application of the timeBAE approach to embryoid body data.** **a** Left: Scatter plot illustrates a 2D UMAP embedding of the PCA representation of the trimmed embryoid body data. Individual cells are colored by their cluster label resulting from Leiden clustering with a resolution parameter of 0.025. Right: Scatter plot shows the same embedding colored by measurement time points, each corresponding to a time interval. **b** Scatter plots of the same UMAP embedding, colored by the representation values of cells in linked timeBAE latent dimensions across time. The first three dimensions are shown. **c** Scatter plots show the top ten normalized nonzero timeBAE encoder weights (represented by linked genes) in decreasing order of absolute value (from left to right) per latent dimension. The first three dimensions are shown. Normalization was performed by dividing each weight by the maximum absolute weight value for the corresponding latent dimension. The first dimension has only seven corresponding nonzero weights.

3 Discussion

Low-dimensional representations of single-cell data allow for visualization of the underlying cellular heterogeneity. We have developed the BAE approach to additionally incorporate structural assumptions, e.g., based on expected patterns or experimental design, directly into the dimension reduction. The approach combines variable selection via componentwise boosting, where the selection criterion can encode constraints reflecting assumptions, with neural network-based autoencoders for dimension reduction. Each latent dimension is associated with a sparse set of genes that characterizes the pattern captured by the variable selection component in that dimension, allowing for a biological interpretation.

To illustrate the flexibility of such an approach for targeted dimension reduction in different scenarios of scRNA-seq analysis, we have developed and illustrated two types of constraints. A constraint for disentangled dimensions reflects the intuitive assumption that different groups of cells (e.g., belonging to the same type or cell stage) are characterized by non-overlapping gene sets, while a constraint capturing different developmental trajectories in time-series scRNA-seq data reflects experimental design knowledge about gradual changes in gene expression patterns between time points. Accordingly, we have applied the BAE approach to capture known marker genes of neurons, reflecting the assumption that distinct groups are present in the data, and to recover differentiation trajectories. The BAE partitioned distinct cell types in uncorrelated latent dimensions along with a sparse set of biologically meaningful marker genes in an application on the subset of neurons from the primary visual cortex of mice, and allowed the recovery of distinct differentiation lineages in simulated and real time series data. While we developed the second constraint to analyze time-series scRNA-seq data, the approach could be more generally applicable to study changes in gene sets of cell groups between conditions, e.g., health and disease.

Notably, the approach simultaneously infers cell groups and characterizing genes, in contrast to standard two-step approaches, where clustering and differential gene expression analysis for marker gene annotation are performed separately. Our joint approach allows even small groups of cells to be identified and characterized by their associated gene sets. In an exemplary application, we were able to resolve the identity of a small group that was not captured by the original annotation, and recover several subtypes of *Sst*-expressing neurons in a subgroup analysis, illustrating the potential of incorporating constraints into scRNA-seq dimension reduction.

Of course, for each specific type of constraint within the BAE approach, such as the sparsity or disentanglement constraint, there may be several alternative approaches. Rather than benchmarking of each constraint variant individually, our focus was on investigating the usefulness of the overall framework for facilitating a biological understanding in different application scenarios. Nevertheless, we provide an exemplary comparison to alternative approaches in Supplementary Section S7. Using the simulation design from [35], we show that the BAE is the only approach that simultaneously provides a sparse and disentangled representation without additional loss function penalty terms.

In the time series example, we subset the dataset so that both cluster and temporal structure could be captured simultaneously in a 2D UMAP visualization, to be able to evaluate whether our approach could correctly identify meaningful structure in a setting where no ground truth annotation was available. In this simplified scenario, a simpler approach might also be able to pick up genes within each cluster that are differentially expressed between time points. While our approach is more generally applicable in more complex settings where the underlying patterns cannot be visualized in 2D, it may then represent multiple lineages in the same set of coupled latent dimensions,

requiring more detailed post-hoc analyses of the identified trajectories.

While the BAE approach is relatively robust to the number of latent dimensions, some intuition about the expected number of different groups in the data is required to set the number of dimensions within a suitable range. In addition, when fitting time series data, we currently assume a constant number of groups over time. To also allow for branching trajectories, which are typical for many differentiation processes, one dimension at an earlier time point could be linked to several dimensions at a later time point, e.g., by tailoring the penalization in the boosting coefficient updates. In addition, the BAE in its current form has a linear encoder, which can limit flexibility and affect reconstruction performance. While we found the simple single-layer encoder sufficient in the applications, an extension to include a non-linear activation function and additional layers is in principle feasible. A non-linear activation function may require to extend the boosting to a generalized linear model framework, as discussed in [43], and choosing an appropriate link function. To include additional layers, our joint optimization strategy based on differentiable programming would allow differentiation through the boosting layer to obtain gradient feedback for the previous layers. However, the boosting coefficients would then not directly correspond to the contribution of individual input variables, but to a (potentially non-linear) combination of them. Feature attribution methods, such as those discussed in [66, 67], could be used to solve this problem.

In our application, we have focused on highly variable genes as model inputs. However, a key advantage of boosting is that it can handle very large numbers of variables [45]. The approach could thus be scaled to even higher-dimensional input data, where approaches from [68] could be employed for computational efficiency. This could also be useful, for example, for single-cell ATAC-sequencing data on chromatin accessibility, which is more sparse and high-dimensional than scRNA-seq data. The approach could then also be extended to a multi-omics setting, where a latent representation is learned that integrates different modalities, and the selection criterion could be adapted to capture modality-specific or modality-overlapping information in different dimensions, e.g. by considering residuals of model fits from one modality for fitting the other modality. Further, additional knowledge about the correspondence between peaks and genes could be incorporated by adjusting the penalty for the corresponding boosting coefficient updates, to increase the probability of selecting these variables together [45, 69]. Similar strategies could be used to incorporate knowledge about pathway structure or gene regulatory networks.

4 Conclusions

Dimension reduction methods perform well on scRNA-seq data, but are difficult to interpret. The BAE approach demonstrates how deep learning and statistical modeling can be integrated to obtain a targeted representation that reflects biologically plausible assumptions and is explained by small sets of genes. Our approach illustrates the potential benefits of combining data-driven and knowledge-driven modeling for scRNA-seq data to take a step towards better understanding of complex biological processes at the single-cell level.

5 Methods

5.1 Mathematical notations

Matrices are written in capital bold letters, and vectors in lower case bold letters. Greek letters are used for denoting model parameters or hyper parameters. Vectors are treated as column vectors.

Scalar values are denoted by lower case letters. In general, $\boldsymbol{X} \in \mathbb{R}^{n \times p}$ denotes a matrix of n i.i.d. observations of p variables sampled from an unknown probability distribution. Observations, i.e., rows of $\boldsymbol{X}$, are denoted by $\boldsymbol{x}_1, \ldots, \boldsymbol{x}_n$ and the columns of $\boldsymbol{X}$ by $\boldsymbol{x}^{(1)}, \ldots, \boldsymbol{x}^{(p)}$. We define the zero-matrix with n rows and p columns by $\mathbf{0}_{\mathbb{R}^{n \times p}}$. Further, by $\widehat{\mu}_{\boldsymbol{x}} := \frac{1}{n} \sum_{i=1}^{n} x_i$ we denote the sample mean of a vector $\boldsymbol{x} = (x_1, \ldots, x_n)^\top$ and by $\widehat{\sigma}_{\boldsymbol{x}} := \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \widehat{\mu}_{\boldsymbol{x}})^2}$ the corrected sample standard deviation of $\boldsymbol{x}$. The sample Pearson correlation coefficient of two vectors $\boldsymbol{x}, \boldsymbol{y}$ is denoted by $\widehat{\mathrm{cor}}(\boldsymbol{x}, \boldsymbol{y}) := \frac{\sum_{i=1}^{n} (x_i - \widehat{\mu}_{\boldsymbol{x}}) \cdot (y_i - \widehat{\mu}_{\boldsymbol{y}})}{\widehat{\sigma}_{\boldsymbol{x}} \widehat{\sigma}_{\boldsymbol{y}}}$. Given a vector $\boldsymbol{x}$ we refer to its standardized version by computing the z-score for each component $\widehat{\boldsymbol{x}} = (\widehat{x}_1, \ldots, \widehat{x}_n)^\top$, where $\widehat{x}_i := \frac{x_i - \widehat{\mu}_{\boldsymbol{x}}}{\widehat{\sigma}_{\boldsymbol{x}}}$, $i = 1, \ldots, n$. Furthermore, given a matrix $\boldsymbol{X} \in \mathbb{R}^{n \times p}$ and a set $\mathcal{I} \subset \{1, \ldots, p\}$ with $|\mathcal{I}| < p$, we denote by $\boldsymbol{X}^{(-\mathcal{I})}$ the sub-matrix consisting of only the columns with indices that are not elements of $\mathcal{I}$. In the special case of $\mathcal{I} = \emptyset$, $\boldsymbol{X}^{(-\mathcal{I})} = \boldsymbol{X}$.

5.2 Deep learning and autoencoders

Deep learning models are based on artificial neural networks (NNs) [70]. Technically, NNs correspond to function compositions of affine functions and so-called activation functions, organized in layers. Formally, for $m, n_0, \ldots, n_m \in \mathbb{N}$ with $m \geq 2$, given some differentiable and typically non-linear functions $\rho^{(i)} : \mathbb{R} \mapsto \mathbb{R}$, weight matrices $\boldsymbol{W}^{(i)} \in \mathbb{R}^{n_{i-1} \times n_i}$ and bias vectors $\boldsymbol{b}^{(i)} \in \mathbb{R}^{n_i}$, $i = 1, \ldots, m$, a NN is a function composition

$$
\begin{aligned}
&f : \mathbb{R}^{n_0} \mapsto \mathbb{R}^{n_m} \\
&f(\boldsymbol{x}) := (f^{(m)} \circ \ldots \circ f^{(1)})(\boldsymbol{x}),
\end{aligned}
$$

where for $i = 1, \ldots, m$ the functions $f^{(1)}, \ldots, f^{(m)}$ represent the individual layers of the NN, defined by

$$
\begin{aligned}
&f^{(i)} : \mathbb{R}^{n_{i-1}} \mapsto \mathbb{R}^{n_i} \\
&f^{(i)}(x) := \rho^{(i)}_{(\cdot)}(\boldsymbol{W}^{(i)} \boldsymbol{x} + \boldsymbol{b}^{(i)}).
\end{aligned}
$$

Thereby, $(\cdot)$ in the subscript of an activation denotes its elementwise application to the input vector.

The deep architecture of NNs allow for modeling complex relationships (e.g. see [71, 72, 73]). The search for optimal weights and biases, i.e. the training of the NN, is usually performed via gradient-based optimization schemes. Therefore, given a fixed observation vector $\boldsymbol{x} \in \mathbb{R}^{n_0}$, a NN can be equivalently considered as a mapping from a finite-dimensional parameter space $\Theta := \mathbb{R}^{n_0 \times n_1} \times \mathbb{R}^{n_1} \times \ldots \times \mathbb{R}^{n_{m-1} \times n_m} \times \mathbb{R}^{n_m}$ to the output space $\mathbb{R}^{n_m}$

$$
\begin{aligned}
&NN : \Theta \mapsto \mathbb{R}^{n_m}, \\
&NN(\boldsymbol{x};\, \boldsymbol{\theta}) := f(\boldsymbol{x};\, \boldsymbol{\theta}) = (f^{(m)}_{\boldsymbol{W}^{(m)}, \boldsymbol{b}^{(m)}} \circ \ldots \circ f^{(1)}_{\boldsymbol{W}^{(1)}, \boldsymbol{b}^{(1)}})(\boldsymbol{x}),
\end{aligned}
$$

where $\boldsymbol{\theta} = (\boldsymbol{W}^{(1)}, \boldsymbol{b}^{(1)}, \ldots, \boldsymbol{W}^{(m)}, \boldsymbol{b}^{(m)})$ represents a tuple of weight matrices and bias vectors.

An autoencoder(AE) is a specific neural network architecture with a bottleneck-like structure frequently used for unsupervised representation learning of high-dimensional data. Specifically, AEs consist of an encoder network, which defines a parametric mapping from the observation space into a lower-dimensional latent space, and a decoder network, which performs the reverse

mapping back to data space, each parameterized by a tuple of tunable weight matrices and bias vectors

$$AE : \Theta \mapsto \mathbb{R}^{n_0},$$
$$AE(\boldsymbol{x};\ (\boldsymbol{\psi}, \boldsymbol{\theta})) := f_{\text{dec}}(f_{\text{enc}}(\boldsymbol{x};\ \boldsymbol{\psi});\ \boldsymbol{\theta}).$$

To learn a low-dimensional representation of the observed data that reflects the main underlying characteristics, the training objective for AEs is to approximate the identity function under the constraint that the function input first gets compressed to a low-dimensional space before the reconstruction. This requires a differentiable scalar valued reconstruction loss function $L_{\text{rec}} : \mathbb{R}^{n_0} \mapsto \mathbb{R}^{n_0}$ which measures the similarity of the reconstructed output to the original input. A common choice for continuous observation data is the mean squared error

$$L_{\text{rec}}(\boldsymbol{x}, \widehat{\boldsymbol{x}}) := \frac{1}{p} \sum_{j=1}^{p} (x_j - \widehat{x}_j)^2.$$

For normally distributed data, minimizing the reconstruction loss, i.e., the mean squared error, then corresponds to maximum likelihood estimation of the network parameters.

Given observations $\boldsymbol{x}_1, \ldots, \boldsymbol{x}_n \in \mathbb{R}^{n_0}$, the search for optimal weight matrices and bias vectors of the AE is performed by iteratively minimizing the empirical risk

$$\underset{(\boldsymbol{\psi}, \boldsymbol{\theta}) \in \Theta}{\arg\min} \frac{1}{n} \sum_{i=1}^{n} L_{\text{rec}}(\boldsymbol{x}_i, AE(\boldsymbol{x}_i;\ (\boldsymbol{\psi}, \boldsymbol{\theta}))), \tag{1}$$

via a gradient-based optimization algorithm such as stochastic gradient descent. Starting with a random initialization of the weight matrices and bias vectors, in each iteration the current parameters are adapted into the direction of the negative gradient of a randomly determined partial sum of the objective in (1) w.r.t. the weight matrices and bias vectors. To avoid making too large steps into the direction of the negative gradients and to stabilize the optimization, the gradients are multiplied by a scalar factor $\nu \in (0, 1)$. An advanced version of stochastic gradient descent is the ADAM optimizer [62], a computationally efficient first-order gradient-based method with an adaptive learning rate incorporating additionally first- and second-order moments of the gradients.

After training an AE, the observations can be mapped to the latent space using the encoder $f_{\text{enc}}(\boldsymbol{x};\ \boldsymbol{\psi})$. As the encoder is a parametric mapping, it can map previously unseen data from the same underlying data distribution as the training data into the latent space.

5.3 Componentwise likelihood-based boosting

In the following we describe generalized likelihood-based boosting with componentwise linear learners (compL_2Boost) [43, 44] (see also [45, 74, 75]). While e.g. in [43] the procedure is described in a more general setting including several versions of generalized linear models, we consider only the case of i.i.d. homoscedastic normally distributed responses, such that the link function corresponds to the identity function. As a result, the version stated here is equivalent to gradient boosting with componentwise linear learners using the squared-error loss [51, 76], and to forward stagewise linear regression [49].

The compL_2Boost algorithm is a stagewise variable selection approach that updates only one of the regression coefficients in each iteration. Consequently, boosting results in a sparse estimate

of the coefficient vector and can produce stable estimates even if the number of variables exceeds the number of observations by far. This makes boosting highly useful and sucessful for high-dimensional omics data, such as from scRNA-seq.

Consider some pairs of observations $(\boldsymbol{x}_i, y_i)_{i=1,\dots,n}$ of covariate vectors $\boldsymbol{x}_i \in \mathbb{R}^p$, defining the matrix $\boldsymbol{X} := (\boldsymbol{x}^{(1)}, \dots, \boldsymbol{x}^{(p)}) := (\boldsymbol{x}_1, \dots, \boldsymbol{x}_n)^\top \in \mathbb{R}^{n \times p}$ and a continuous response vector $\boldsymbol{y} = (y_1, \dots, y_n)^\top \in \mathbb{R}^n$. The responses y_i are assumed to be realizations of independent homoscedastic random variables $Y_i \sim \mathcal{N}((\boldsymbol{x}_i)^\top \boldsymbol{\beta}, \sigma^2)$ for a fixed $\sigma^2 > 0$ and an unknown coefficient vector $\boldsymbol{\beta} \in \mathbb{R}^p$, which is to be estimated. In the following, we assume that the covariate vectors $\boldsymbol{x}^{(j)},\ j = 1, \dots, p$ are standardized, i.e., $\hat{\mu}_{\boldsymbol{x}^{(j)}} = 0$ and $\sum_{i=1}^n x_{ij}^2 = n - 1$ using the corrected standard deviation estimator.

In the beginning of the algorithm, regression coefficients $\widehat{\boldsymbol{\beta}}^{(0)} := (0, \dots, 0)^\top$ are initialized at 0 and we define the initial offset $\widehat{\boldsymbol{\eta}}^{(0)} := (\widehat{\eta}_1^{(0)}, \dots, \widehat{\eta}_n^{(0)})^\top = (0, \dots, 0)^\top$. For a fixed step size $\varepsilon \in (0, 1)$ and a fixed number of boosting steps $M \in \mathbb{N}$, the updates for the regression coefficients in every step $m = 1, \dots, M$ are computed as follows:

1) For each component $j = 1, \dots, p$, define the linear predictors

$$\eta_{i,j}^{(m)} = \widehat{\eta}_i^{(m-1)} + \gamma_j^{(m)} x_{ij},\ i = 1, \dots, n,$$

for which we want to determine the index j^* resulting in the best fit at the current iteration.

2) For $j = 1, \dots, p$, compute the maximum likelihood estimator $\widehat{\gamma}_j^{(m)}$ of the (partial) log-likelihood

$$l(\gamma_j^{(m)}) := \log(\prod_{i=1}^n p(y_i \mid (\boldsymbol{x}_i, j))) = -\frac{n}{2}\log(2\pi\sigma^2) - \frac{1}{2\sigma^2}\sum_{i=1}^n((y_i - \widehat{\eta}_i^{(m-1)}) - \gamma_j^{(m)} x_{ij})^2, \quad (2)$$
$$y_i \mid (\boldsymbol{x}_i, j) \sim \mathcal{N}(\eta_{i,j}^{(m)}, \sigma^2).$$

Assuming that at least one entry of each covariate vector differs from zero and since only the second summand of the right side of Equation (2) depends on $\gamma_j^{(m)}$, the maximizer for each index $j = 1, \dots, p$ can be derived by calculating the unique root of the derivative

$$\frac{\partial\, l(\gamma_j^{(m)})}{\partial\, \gamma_j^{(m)}} = \frac{1}{\sigma^2}(\boldsymbol{x}^{(j)})^\top((\boldsymbol{y} - \widehat{\boldsymbol{\eta}}^{(m-1)}) - \gamma_j^{(m)} \cdot \boldsymbol{x}^{(j)}),$$
$$\widehat{\gamma}_j^{(m)} = \frac{(\boldsymbol{x}^{(j)})^\top(\boldsymbol{y} - \widehat{\boldsymbol{\eta}}^{(m-1)})}{(\boldsymbol{x}^{(j)})^\top \boldsymbol{x}^{(j)}}.$$

The j-th solution equals the score function $S(\gamma_j^{(m)}) = \frac{\partial\, l(\gamma_j^{(m)})}{\partial\, \gamma_j^{(m)}}$ of the log-likelihood divided by the fisher information $I(\gamma_j^{(m)}) = -\frac{\partial^2\, l(\gamma_j^{(m)})}{\partial\, (\gamma_j^{(m)})^2}$ both evaluated at $\gamma_j^{(m)} = 0$. Hence, $\widehat{\gamma}_j^m$ can be considered as the result of one fisher scoring step with starting point at zero [43].

3) Out of all the computed maximum likelihood estimators in the current iteration, determine

the one $\widehat{\gamma}_{j^*}^{(m)}$ with the highest score

$$
\begin{aligned}
j^* &= \underset{j=1,\dots,p}{\arg\max} \frac{\left(S(\gamma_j^{(m)})|_{\gamma_j^{(m)}=0}\right)^2}{I(\gamma_j^{(m)})|_{\gamma_j^{(m)}=0}} \\
&= \underset{j=1,\dots,p}{\arg\max} \left((\boldsymbol{x}^{(j)})^\top(\boldsymbol{y} - \widehat{\boldsymbol{\eta}}^{(m-1)})\right)^2.
\end{aligned}
$$

Note that the denominator is constant for standardized covariate vectors and can thus be neglected as it is independent of j.

4) Compute the updated coefficient vector by

$$
\widehat{\boldsymbol{\beta}}^{(m)} = \begin{cases} \widehat{\beta}_j^{(m-1)} + \varepsilon\widehat{\gamma}_j^{(m)} & , j = j^* \\ \widehat{\beta}_j^{(m-1)} & , \text{else.} \end{cases}
$$

and define the new offset $\widehat{\boldsymbol{\eta}}^{(m)} = \widehat{\boldsymbol{\eta}}^{(m-1)} + \varepsilon\widehat{\gamma}_{j^*} \cdot \boldsymbol{x}^{(j)}$.

The sparsity level of the final coefficient vector is directly regulated by the number of boosting steps where for $M \in \mathbb{N}$ boosting steps, at most M coefficients can become nonzero. Additionally, the step size ε influences the sparsity level, where either making too large steps or to small steps increases the sparsity of the coefficient vector.

A pseudocode for the version of the compL_2Boost algorithm that we utilize in the optimization process of the BAE (see Methods Section 5.4) for standardized covariate vectors and a mean centered response vector is stated in Algorithm 1 in the Appendix.

5.4 Core optimization algorithm of the BAE

A BAE is an autoencoder with a specific architecture, where compL_2Boost is integrated into the gradient-based optimization scheme for the training. While the decoder of a BAE can be chosen arbitrarily, the encoder consists of only a linear layer

$$
\begin{aligned}
&BAE : \Theta \mapsto \mathbb{R}^p, \\
&BAE(\boldsymbol{x};\ (\boldsymbol{B}, \boldsymbol{\theta})) := f_{\text{dec}}(f_{\text{enc}}(\boldsymbol{x};\ \boldsymbol{B});\ \boldsymbol{\theta}), \\
&f_{\text{enc}}(\boldsymbol{x};\ \boldsymbol{B}) := \rho_{(\cdot)}^{\text{enc}}(\boldsymbol{B}^\top \boldsymbol{x}), \\
&\rho^{\text{enc}}(x) := \mathrm{id}(x),
\end{aligned}
$$

where $\boldsymbol{B}$ denotes the transposed encoder weight matrix. Note that extensions to more complex encoder architectures are possible, as discussed in the Discussion Section 3.

Starting with a zero-initialized encoder weight matrix and randomly initialized decoder parameters, the optimization of the BAE in each training epoch is performed in an alternating manner by optimizing the encoder weights via successive application of the compL_2Boost algorithm to each latent dimension and optimization of the decoder parameters using a gradient-based optimization algorithm such as stochastic gradient descent or ADAM. To integrate boosting as a supervised approach into an unsupervised optimization process, we define pseudo responses to update the linear model coefficients, corresponding to the rows of the encoder weight matrix.

Consider observations $\boldsymbol{X} \in \mathbb{R}^{n \times p}$ with standardized covariate vectors and a BAE with $d \ll p$ latent dimensions. Due to standardization, we work with normality assumptions and thus choose the mean squared error as the reconstruction loss:

$$L_{\text{rec}}(\boldsymbol{x}, \widehat{\boldsymbol{x}}) := \frac{1}{p} \sum_{j=1}^{p} (x_j - \widehat{x}_j)^2.$$

In an arbitrary training epoch $k \in \mathbb{N}$, let $\boldsymbol{B} = (\boldsymbol{\beta}^{(1)}, \ldots, \boldsymbol{\beta}^{(d)}) \in \mathbb{R}^{p \times d}$ denote the current transposed encoder weight matrix, where each column vector $\boldsymbol{\beta}^{(l)}$ represents the current regression coefficients and $\boldsymbol{Z} = (\boldsymbol{z}^{(1)}, \ldots, \boldsymbol{z}^{(d)}) := \boldsymbol{X}\boldsymbol{B}$ represents the latent representation, consisting of the current prediction vectors $\boldsymbol{z}^{(l)}$, for each latent dimension $l = 1, \ldots, d$. The first step for updating the encoder weights is to compute the negative gradients of the training objective with respect to the prediction vectors $\boldsymbol{z}^{(l)}$ as pseudo responses, defining

$$\boldsymbol{g}^{(l)} := -\nabla_{\boldsymbol{z}^{(l)}} \frac{1}{n} \sum_{i=1}^{n} L_{\text{rec}}(\boldsymbol{x}_i, f_{\text{dec}}(\boldsymbol{z}_i;\ \boldsymbol{\theta})),\ l = 1, \ldots, d.$$

The negative gradients represent the directions in which the predictions have to be adjusted to minimize the training objective the most. We standardize the pseudo responses to fulfill the required normality assumption for the compL_2Boost from Subsection 5.3, and to omit an intercept term

$$\widehat{\boldsymbol{g}}^{(l)} = (\widehat{g}_1^{(l)}, \ldots, \widehat{g}_n^{(l)})^\top,\ \widehat{g}_i^{(l)} := \frac{g_i^{(l)} - \widehat{\mu}_{\boldsymbol{g}^{(l)}}}{\widehat{\sigma}_{\boldsymbol{g}}^{(l)}},\ i = 1, \ldots, n.$$

Note that after standardization, the pseudo responses still define descent directions for the training objective. Subsequently, updates for the regression coefficients, i.e., the weights of the encoder, are obtained via successively applying the compL_2Boost algorithm for only one boosting step with a step size $\varepsilon \in (0, 1)$ to the pairs of observations and pseudo responses $(\boldsymbol{x}_i, \widehat{g}_i^{(l)})_{i=1}^n$

$$\boldsymbol{\beta}^{(l)} = \text{comp}L_2\text{Boost}(\boldsymbol{\beta}^{(l)}, \boldsymbol{X}, \widehat{\boldsymbol{g}}^{(l)};\ \varepsilon, \text{steps=1}),\ l = 1, \ldots, d.$$

Subsequently, the decoder parameters are updated by performing one step of a gradient-based optimization scheme.

After training, the latent representation can be computed by feeding the data to the trained encoder f_{enc}, parameterized by the sparse encoder weight matrix. Each nonzero weight corresponds to a link between a latent dimension and one of the input variables. At the same time, its absolute value is an indicator of the importance of that variable.

Algorithm 2 in the Appendix provides a pseudocode of the alternating core BAE optimization algorithm. We refer to this alternating optimization procedure as optimization in alternating mode. Although described in an alternating manner here, the gradients of the training objective w.r.t. $\boldsymbol{z}^{(1)}, \ldots, \boldsymbol{z}^{(d)}$ and the decoder parameters could be computed simultaneously on the same training batches. This would allow for parallelization of the subsequent update computations using different optimization criteria.

Alternatively, encoder and decoder can be trained jointly, which simplifies training by using an end-to-end instead of a two-step strategy, and allows for adding further layers to the encoder for capturing more complex patterns. This requires to differentiate through the boosting step in each

epoch to obtain gradients of the reconstruction loss that respect the encoder parameter updates, which can be realized efficiently using differentiable programming [77] and a flexible automatic differentiation framework [53] (see Methods Section 5.6).

Note that the activation functions need to be selected such that their derivatives do not vanish at zero, which happens, e.g., for standard implementations of the popular ReLU function. Otherwise the pseudo responses vanish and the encoder weight matrix stays zero during the entire training process.

Applying compL_2Boost for only one boosting step to each latent dimension results in a coarse approximation of the current pseudo responses in each training epoch, where only one element of the associated row vector of the encoder weight matrix is updated. However, applying the algorithm for only one step is sufficient, as the fit is carried out as part of an iterative optimization process. In general, by training a BAE as described above, with d latent dimensions for a number of N training epochs, at least d elements but at most $d \cdot N$ elements of the encoder weight matrix become nonzero during the training. Consequently, the sparsity level of the encoder weight matrix is highly influenced by the number of training epochs and boosting steps. For reaching a desired sparsity level, we suggest limiting the maximum number of nonzero coefficients per dimension by training for a fixed number of epochs while adjusting the step size for the boosting component, regulating the number of nonzero coefficients. We believe that introducing regularization for the decoder parameters and the coefficients of the boosting component (see e.g. in [45, 69]) could further stabilize the variable selection when training for a large amount of epochs and prevent it from overfitting.

Yet, generally tuning of componentwise boosting is relatively robust, e.g., if training is continued beyond the necessary number of boosting steps, mostly parameters of already selected genes will receive small updates that do not significantly alter the representation (see also Supplementary Section S7, Supplementary Figure S8). Throughout our applications, an expressive set of variables was selected already during the very first training epochs.

To illustrate the optimization process of the BAE and verify its effectiveness in representing distinct cell groups and identifying characteristic sparse gene sets in each latent dimension via modification of the selection criterion, we provide a simple simulation design in Supplementary Section S6 and in a Jupyter notebook. A comparison to potential alternative approaches using regularization techniques is provided in Supplementary Section S7.

5.5 Disentanglement constraint for structuring latent representations

So far, we have explained the BAE optimization using unconstrained gradients as pseudo responses. To learn disentangled latent dimensions, we constrain the pseudo responses for the boosting in the encoder optimization. Consider the same setting as described in the previous Subsection 5.4 for updating the encoder weight matrix. For each latent dimension $l = 1, \ldots, d$, instead of taking the standardized negative gradient $\widehat{\boldsymbol{g}}^{(l)}$ as the pseudo responses, only the fraction of the gradient that is currently not explainable by the other latent dimensions $k \in \{k = 1, \ldots, d \mid k \neq l\}$ is used as the constrained pseudo responses. Therefore, we first define by

$$\mathcal{I} := \{l\} \cup \{k = 1, \ldots, d \mid \beta^{(k)} = \mathbf{0}_{\mathbb{R}^p}\}$$

the set of all indices of latent dimensions with corresponding all-zero encoder weights together with the current index l. Note that zero weight vectors only occur in the first training epoch. The matrix $\boldsymbol{Z}^{(-\mathcal{I})}$ then consists of only the columns of $\boldsymbol{Z}$ whose indices do not appear in $\mathcal{I}$. Exclusion of

zero columns is important for avoiding technical invertibility problems in the following computations. Next, we predict the best linear fit of $\boldsymbol{Z}^{(-\mathcal{I})}$ to the pseudo responses $\widehat{\boldsymbol{g}}^{(l)}$ by first computing the ordinary linear least squares estimator

$$\begin{aligned}\boldsymbol{\alpha}^* :&= \underset{\boldsymbol{\alpha}\in\mathbb{R}^{d-|\mathcal{I}|}}{\arg\min}\ \frac{1}{2}\|\widehat{\boldsymbol{g}}^{(l)} - \boldsymbol{Z}^{(-\mathcal{I})}\boldsymbol{\alpha}\|_2^2 \\ &= ((\boldsymbol{Z}^{(-\mathcal{I})})^\top \boldsymbol{Z}^{(-\mathcal{I})})^{-1}(\boldsymbol{Z}^{(-\mathcal{I})})^\top \widehat{\boldsymbol{g}}^{(l)},\end{aligned}$$

and subsequently calculating the residual vector

$$\boldsymbol{y}^{(l)} := \widehat{\boldsymbol{g}}^{(l)} - \boldsymbol{Z}^{(-\mathcal{I})}\boldsymbol{\alpha}^*.$$

The constrained pseudo responses for the boosting are then defined as the standardized vector

$$\widehat{\boldsymbol{y}}^{(l)} = (\widehat{y}_1^{(l)}, \dots, \widehat{y}_n^{(l)})^\top,\ \widehat{y}_i^{(l)} := \frac{y_i^{(l)} - \widehat{\mu}_{\boldsymbol{y}^{(l)}}}{\widehat{\sigma}_{\boldsymbol{y}^{(l)}}},\ i = 1, \dots, n,$$

representing the fraction of the original pseudo responses $\widehat{\boldsymbol{g}}^{(l)}$ that is currently not explained by other latent dimensions $k \in \{k = 1, \dots, d \mid k \neq l\}$.

In the special case of updating $\boldsymbol{\beta}^{(1)}$ during the first training epoch, where no variables have been selected yet, $\mathcal{I} = \{1, \dots, d\}$. In that case, we neglect the least squares estimation-step and directly set $\widehat{\boldsymbol{y}}^{(1)} = \widehat{\boldsymbol{g}}^{(1)}$.

A pseudocode for the disentanglement constraint that can be included in the core optimization algorithm for adapting the pseudo response of each latent dimension in each training epoch is stated in Algorithm 2 in the Appendix.

5.6 Differentiable programming for joint optimization

For a better communication between the encoder and decoder parameter updates, we provide an alternative optimization procedure for the BAE. While in the optimization process described in 5.4 updates for the encoder- and decoder parameters are performed in an alternating way, they can also be optimized simultaneously using differentiable programming [77, 53, 52], a paradigm for flexibly coupling optimization of diverse model components. For an overview from a statistical perspective, see, e.g., [78]. Specifically, we incorporate the boosting part of the optimization as part of a recursively defined joint objective $L_{\text{joint}}(\boldsymbol{X};\ \boldsymbol{\theta})$

$$\begin{aligned}&\widehat{\boldsymbol{B}}_{\boldsymbol{\theta}} = (\text{comp}L_2\text{Boost}(\boldsymbol{\beta}^{(l)}, \boldsymbol{X}, \widehat{\boldsymbol{g}}_{\boldsymbol{\theta}}^{(l)};\ \varepsilon, \text{steps} = 1))_{l=1,\dots,d}, \\ &\widehat{\boldsymbol{Z}}_{\boldsymbol{\theta}} = \boldsymbol{X}\widehat{\boldsymbol{B}}_{\boldsymbol{\theta}}, \\ &L_{\text{joint}}(\boldsymbol{X};\ \boldsymbol{\theta}) = \frac{1}{n}\sum_{i=1}^{n} L_{\text{rec}}(\boldsymbol{x}_i,\ f_{\text{dec}}(\widehat{\boldsymbol{Z}}_{\boldsymbol{\theta}};\ \boldsymbol{\theta})).\end{aligned}$$

Hence, updates for the decoder parameters respect changes in the encoder parameters. Thereby, the $\boldsymbol{\theta}$ subscript indicates the dependence on the current state of the decoder parameters. This procedure is facilitated by differentiable programming, which allows for jointly optimizing the neural network and boosting component by providing gradients of a joint loss function with respect to the parameters of all components via automatic differentiation. In particular, we use the Zygote.jl

automatic differentiation framework [53], which allows for differentiating through almost arbitrary code without user refactoring.

By each call to the joint objective, when optimizing the decoder parameters in one training epoch via an iterative gradient-based optimization algorithm, boosting is performed in the forward pass, hence, the encoder weights are updated in the forward pass.

The joint version of the objective allows to adapt our previously described alternating optimization algorithm to a stochastic framework, meaning that the joint objective can also be applied to randomly sampled mini-batches consisting of $m \leq n$ of the observation vectors. This enables the simultaneous optimization of the encoder and decoder parameters using the same mini-batch. Thus, the boosting part of the BAE optimization process acts on mini-batches, which is related to the stochastic gradient boosting approach described in [79].

Note that using the joint objective function for the optimization leads to a slight increase in training duration since the calculation of the reconstruction loss also incorporates the boosting step. Also, depending on the implementation of the mini-batch procedure, each training epoch could consist of updating all model parameters iteratively, using a partition of the whole dataset into distinct mini-batches of size m. Consequently, the boosting component could be applied several times per epoch, which affects the sparsity level of the encoder weight matrix.

We refer to this joint optimization procedure as optimization in jointLoss mode.

5.7 Adaptation for time series

As an additional use case, we illustrate an adaption of the BAE for handling time series data. Therefore, consider a N-tuple consisting of snapshots of observation data at different time points $(\boldsymbol{X}^{(1)}, \ldots, \boldsymbol{X}^{(N)})$. E.g., these could be scRNA-seq datasets from the same tissue sequenced at different time points. The matrices might contain different numbers of observations/cells, i.e. $\boldsymbol{X}^{(i)} \in \mathbb{R}^{n_t \times p}$, for $n_1, \ldots, n_N \in \mathbb{N},\ t = 1, \ldots, N$.

We assume that the data reflects a developmental process of cells across measurement time points. E.g., cells differentiate into different (sub-)types, and each process is characterized by a small gene set that gradually changes over time by sequential up- and downregulation of genes along the developmental trajectory. For each developmental process, we aim to capture the changes in the associated gene set in linked latent dimensions, one for each time point in an adapted version of the BAE, called timeBAE.

More precisely, the timeBAE comprises one BAE for each time point. The models are trained successively with the disentanglement constraint (see Subsections 5.4 and 5.5) for a fixed number of epochs on data from the corresponding time point using a pre-training strategy. At each time point, the encoder weight matrix from the previous time point is used for initializing the current encoder weight matrix. Once the complete training process is done, we concatenate all the encoder weight matrices and end up with a new stacked encoder weight matrix with $d \cdot N$ latent dimensions. For each $k = 1, \ldots, d$, dimensions $k + (t-1)d$ for $t = 1 \ldots N$ then represent the same developmental process across time. This requires some prior knowledge about the expected number $d \in \mathbb{N}$ of developmental processes of cell groups.

For a formal description, consider N BAEs with linear encoders $f_{\text{enc}}^{(N)}(\boldsymbol{x};\ \boldsymbol{B}^{(N)}) := (\boldsymbol{B}^{(N)})^\top \boldsymbol{x}$, where at the beginning of the training, $\boldsymbol{B}^{(0)} := \boldsymbol{0}_{\mathbb{R}^{p \times d}}$. Each BAE is trained with the data from the respective time point. First, decoder parameters are initialized randomly. We denote the updated matrix obtained after training at time t by $\boldsymbol{B}^{(t)}$, and the next initialization of the encoder weight matrix at time $t+1$ by $\widetilde{\boldsymbol{B}}^{(t+1)}$. We set $\widetilde{\boldsymbol{B}}^{(1)} := \boldsymbol{B}^{(0)}$. Specifically, for the following time points we

set

$$\widetilde{\boldsymbol{B}}^{(t+1)} := \boldsymbol{B}^{(t)} - \widetilde{\boldsymbol{B}}^{(t)},\ t = 1, \ldots, N.$$

Thus, changes in previous encoder weights are incorporated as prior knowledge guiding the model to select genes corresponding to the same developmental program in the same dimension across time points. After training, the overall transposed encoder weight matrix is defined as $\boldsymbol{B} := (\widetilde{\boldsymbol{B}}^{(2)}, \ldots, \widetilde{\boldsymbol{B}}^{(N+1)}) \in \mathbb{R}^{p \times d \cdot N}$, and the joint encoder as $f_{\text{enc}}(\boldsymbol{x}; \boldsymbol{B}) := \boldsymbol{B}^\top \boldsymbol{x}$.

Note that this approach is compatible with both training in alternating and in jointLoss mode.

5.8 Clustering analysis of BAE and PCA based representations of cortical neurons

We trained a BAE with ten latent dimensions using 20 different decoder parameter initializations in alternating mode with the disentanglement constraint on cortical neurons stemming from the primary visual cortex of mice. After training, we computed the corresponding 20 latent representations of the data. On the other hand, we computed the PCA representation of the data where we only kept the first ten principal components equaling the number of latent dimensions of the BAE. Since PCA is a deterministic approach, we performed Leiden clustering for 20 seeds, resulting in 20 different PCA-clusterings. In contrast, we performed Leiden clustering once for each BAE representation.

Subsequently, we evaluated and compared the clustering results using two popular metrics for cluster analysis, the silhouette score and the adjusted Rand index (e.g., see [80, 81, 82]). The silhouette score is a general quality measure for a single clustering, which is defined as the mean of silhouette coefficients of the clustering. For each observation, the corresponding silhouette coefficient measures how well the observation fits into its cluster compared to others. The silhouette coefficient takes values in $[-1, 1]$. A value of one corresponds to an observation fitting well to its assigned cluster compared to other clusters, and a value of -1 means that the observation fits better to other clusters compared to its own. The adjusted Rand index is a version of the Rand index that is corrected for random chance measuring the similarity of two clusterings. A value of one means that both clusterings are identical, whereas a value of zero corresponds to random labeling. We used the scikit-learn implementation for both clustering metrics.

We report the median silhouette score of the 20 clusterings for each method together with the interquartile ranges of silhouette scores, as well as the median adjusted Rand index, for quantitatively comparing the clustering results (see Results Section 2.2).

5.9 Implementation details

All code to run the model and reproduce all analysis results and figures is publicly available at `https://github.com/NiklasBrunn/BoostingAutoencoder`, including documentation and a tutorial in a Jupyter notebook using the Julia programming language, illustrating the functionality of the approach in a simulation study (see Supplementary Section S6).

The BAE and its modification for analyzing time series data are implemented in the Julia programming language [83], v1.6.7, using the packages CSV (v0.10.11), ColorSchemes (v3.24.0), DataFrames (v1.3.5), Distributions (v0.25.100), Flux (v0.12.1), GZip (v0.5.2), Plots (v1.24.3), ProgressMeter (v1.9.0), StatsBase (v0.33.21), UMAP (v0.1.10), VegaLite (v2.6.0), XLSX (v0.10.0). Scripts for downloading and preprocessing the embryoid body data and parts of the preprocessing of the cortical mouse data are implemented in Python v3.10.11. We added a YAML file to our

GitHub repository containing the information about the used packages and corresponding versions. Users can create a conda environment for running the Python code from the YAML file.

In all our experiments, the encoder of any BAE is defined by

$$f_{\text{enc}}(\boldsymbol{x};\ \boldsymbol{B}) = \boldsymbol{B}^\top \boldsymbol{x},$$

where the matrix $\boldsymbol{B} \in \mathbb{R}^{p \times d}$ denotes the transposed encoder weight matrix and we used 2-layered decoders

$$f_{\text{dec}}(\boldsymbol{z}; (\boldsymbol{W}^{(1)}, \boldsymbol{b}^{(1)}, \boldsymbol{W}^{(2)}, \boldsymbol{b}^{(2)})) = \boldsymbol{W}^{(2)} \text{tanh}_{(\cdot)}(\boldsymbol{W}^{(1)}\boldsymbol{z} + \boldsymbol{b}^{(1)}) + \boldsymbol{b}^{(2)},$$

where $\boldsymbol{W}^{(1)} \in \mathbb{R}^{p \times d}$, $\boldsymbol{b}^{(1)} \in \mathbb{R}^p$, $\boldsymbol{W}^{(2)} \in \mathbb{R}^{p \times p}$ and $\boldsymbol{b}^{(2)} \in \mathbb{R}^p$ denote the trainable weight matrices and bias vectors.

The number of units in the input and output layers corresponds to the number of variables in the datasets (i.e., $p = 1500$ for the cortical mouse data analysis and subgroup analysis, $p = 80$ in the time series simulation, $p = 263$ on the embryoid body dataset, and $p = 50$ in the stair-like simulation design from Supplementary Section S6). The number of latent dimensions was selected according to a rough estimate of the number of groups present or expected in the datasets. Specifically, we used $d = 10$ latent dimensions for the cortical mouse data comprising cells of 15 neuron cell types, $d = 4$ for the subgroup analysis of *Sst*-neurons, $d = 3$ in the time series simulation where we simulated three groups of developing cells, $d = 4$ for the embryoid body data where we obtained three clusters from Leiden clustering with a resolution parameter of 0.025, and $d = 10$ in the stair-like simulation design where we simulated ten groups of cells.

In the boosting part of the optimization, we set the number of boosting steps to $M = 1$ and the step size to $\varepsilon = 0.01$ for all experiments. Specifically, we applied BAEs using the disentanglement constraint in alternating mode to obtain the presented results for the embryoid body data, the clustering analysis on cortical mouse data and the stair-like simulation design, and in jointLoss mode for obtaining the results for the remaining datasets. We chose $m = 500$ as the common batch size for optimizing encoder and decoder parameters for the cortical mouse data, $m = 95$ for application on the *Sst*-neurons, $m = 198$ for the simulated time series scRNA-seq data, $m = 600$ as the batchsize for decoder parameter optimization for the embryoid body data, and $m = 800$ as the batchsize for decoder parameter optimization for the stair-like simulation design. In every scenario, we used the ADAM optimizer [62] for updating the decoder parameters, where the learning rate was set to $\nu = 0.01$. The number of training epochs was set to 25 for obtaining the here presented results on the cortical mouse data, 20 for the *Sst*-neurons, 9 for training the timeBAE with data at each time point of the simulated scRNA-seq data, 15 for training with data at each time point of the embryoid body data, and 15 for the stair-like simulation design.

5.10 Simulation of scRNA-seq-like data with time structure

To simulate scRNA-seq-like data with time structure representing a developmental process of three different cell groups across 3 time points, we followed the simulation design presented in [35] (see also Supplementary Section S5). We generated one binary count matrix for every time point representing a snapshot of the current state of the three cell groups. To enable a more realistic scenario, the data at each time point consists of a different number of cells and $p = 80$ genes. Specifically, a cell taking the value 1 for some gene indicates a gene expression level exceeding a certain threshold for that cell, whereas the value 0 indicates an expression level below that threshold. The data

at each time point is modeled by i.i.d. random variables $(X_{i,j} \sim \text{Ber}(p_{i,j}))_{i=1,\ldots,n,\ j=1,\ldots,p}$, where $p_{i,j}$ denotes the probability of a value of 1 for a gene j in a cell i. We set $p_{i,j}$ to 0.6 for highly expressed genes (HEGs) and to 0.1 for lowly expressed genes (LEGs). To model a smooth transition between two successive cell stages across subsequent time points, we include overlaps between the sets of HEGs from two subsequent time points. Table 1 comprises the details of the gene expression patterns and overlap patterns for the count matrices at different time points. In total there are 54 HEGs for the different cell stages and 26 genes that are lowly expressed in all cell stages across all time points that are added as non-informative genes to make the variable selection task more challenging and realistic. For simplicity, we strictly excluded intersections between the sets of HEGs of distinct cell groups. An exemplary visualization of simulated time series scRNA-seq

	Cell group 1 ($n = 310$)	Cell group 2 ($n = 298$)	Cell group 3 ($n = 306$)
HEGs (time point 1)	1-8	21-28	41-48
HEGs (time point 2)	6-13	26-33	46-53
HEGs (time point 3)	11-18	31-38	51-58

Table 1: **Highly expressed gene patterns in simulated time series scRNA-seq data**

data can be found in Figure 4a.

5.11 Data preprocessing

For the adult mouse cortical scRNA-seq data [61], we downloaded the expression data consisting of the measured gene expression levels of 15,119 genes in 1,679 single cells, the gene names, and cell type annotations from GEO under accession number GSE71585. Next we removed the samples which have been flagged by [61] as being of bad quality. As the quantification of aligned reads per gene has been conducted with RSEM [84], decimal numbers might show up occasionally in the estimated counts per gene. Next, we normalized the data using the DESeq2 normalization for sequencing depth [85] to adjust for fluctuations in the number of aligned reads per sample, affecting each gene's estimated expression level. Afterwards, we log-transformed the normalized counts with an added pseudo-count of one. Genes were removed if count values did not exceed ten in more than 20 cells. Our analysis focused on the subset of cortical neurons based on the cell type annotation provided by [61]. We removed genes expressed in less than ten cells for the remaining cells. Further, we reduced the set of genes to 1,500 highly variable genes, including marker genes of neural cell types (Figure 3 in [61]) and neurotransmitter receptor genes (Supplementary Material, Figure S15 in [61]) that were previously identified by [61] (Figure 3 in [61]). The resulting count matrix consists of 1,525 neurons and 1500 highly variable genes, including 90 of the 180 marker and receptor genes identified by [61].

In the subgroup analysis of cells highly expressing the marker *Sst*, we first selected only the *Sst*-neurons. Subsequently, we removed genes expressed in less than ten cells, followed by highly variable gene selection. The resulting count matrix consists of 202 *Sst*-neurons with gene counts of 1,500 highly variable genes.

The embryoid body data comprises gene expression levels of embryonic cells generated over a 27 day time course, stored in five different count matrices depending on measurement time. Especially, cells sequenced at day 0-3 were labeled as time point 1, cells from day 6-9 as time point 2, cells from day 12-15 as time point 3, cells from day 18-21 as time point 4, and cells from day 24-27 as time point 5. We excluded cells from time point 1 since their expression patterns are mostly

homogeneous. For the remaining cells, we followed and adapted the preprocessing steps from [65]. First, we filtered cells by library size to remove doublets and empty droplets, where for each time point cells below the lower 20th percentile and above the upper 20th percentile were excluded. Next, we filtered genes expressed in less than ten cells, separately for each time point. We further excluded apoptotic cells expressing a large total amount of mitochondrial genes The threshold was set to 400. Subsequently, we normalized the counts for each cell by dividing each count value by the total number of counts across genes. Next, we re-scaled the normalized counts by a factor of 10,000, followed by a log-transformation with a pseudo-count of one. For the timeBAE analysis, we fused the data matrices from time points 3 and 4, creating the new time point 34 for those cells, and subset the data to cells that can be grouped in large clusters. Therefore, we performed PCA and chose the first 50 principal components to apply the Leiden clustering algorithm with resolution parameters of 0.025 and 0.25. Under observation of the clustering results with the resolution parameter 0.25 in a 2D UMAP plot of cells, we then removed clusters that were separated from the larger groups. Next, we selected genes that were differentially expressed between different clusters of the clustering with resolution parameter 0.025. For each cluster we selected genes differentially expressed between the adapted time points. After preprocessing and filtering, we ended up with 11,628 cells and 263 differentially expressed genes.

Since the BAE approach requires observation data in a standardized form, z-scores of the gene vectors were computed for each of the simulated and real-world scRNA-seq datasets. We used the log-transformed counts to standardize the mouse cortical scRNA-seq data and the embryoid body scRNA-seq data. For the simulated scRNA-seq datasets, we re-scaled the noise variables after standardization by multiplying them with a scalar factor to adjust the range of the values to that of the other gene vectors. The scaling factor for the simulated scRNA-seq data with time structure was set to 0.5, and for the stair-like scRNA-seq data for the analysis of the BAE functionality in Supplementary Section S6 to $\frac{2}{3}$.

Declarations

Ethics approval and consent to participate

Not applicable. No human data was used in this study. The animal datasets are publicly available, details are provided in Section 5.11 and below.

Consent for publication

Not applicable.

Availability of data and materials

The scRNA-seq datasets used in this study are publicly available. The adult mouse cortical dataset from [61] can be accessed from the Gene expression Omnibus (GEO) at `https://www.ncbi.nlm.nih.gov/geo/` under the accession number GSE71585 (GSE71585_RefSeq_counts.csv.gz) and the embryoid body scRNA-seq data is available from the Mendeley Data repository at `https://data.mendeley.com/datasets/v6n743h5ng/1`.

Code for the simulated data, for preprocessing and the proposed algorithm is available at `https://github.com/NiklasBrunn/BoostingAutoencoder`, including a tutorial Jupyter notebook

to illustrate the approach and scripts to reproduce all figures and analysis results shown in the manuscript. Further details on the implementation can be found in the Methods Section 5.9.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HB proposed the original idea and supervised the project. NB and MH implemented the approach and designed the computational experiments. NB performed the computational analyses. NB and MH wrote the manuscript. TV aided in the biological interpretation and in revising the manuscript. All authors revised and approved the final manuscript.

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